



## INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

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<b>(54) Title:</b> METHOD FOR MANUFACTURING FAT-SOLUBLE PHYTOSTEROL OR PHYTOSTANOL ESTER OF UNSATURATED FATTY ACID		
<b>(57) Abstract</b> <p>The present invention provides a method for manufacturing fat-soluble phytosterol or phytostanol ester of unsaturated fatty acid for inhibiting the absorption of cholesterol and foodstuffs containing the same. The method for manufacturing fat-soluble phytosterol or phytostanol ester of unsaturated fatty acid comprises the steps of: esterification of phytosterol or phytostanol with unsaturated fatty acid by dissolving them in a nonpolar organic solvent with a basic catalyst and adding a carboxyl group activating agent dissolved in a nonpolar organic solvent; and, precipitation of the esterified product in methanol or a mixture of methanol and acetone, discarding the remaining solvent, and drying the product under a reduced pressure.</p>		

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**METHOD FOR MANUFACTURING FAT-SOLUBLE PHYTOSTEROL OR  
PHYTOSTANOL ESTER OF UNSATURATED FATTY ACID**

BACKGROUND OF THE INVENTION

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Field of the Invention

The present invention relates to a method for manufacturing fat-soluble phytosterol or phytostanol ester of unsaturated fatty acid for inhibiting the absorption of cholesterol and foodstuffs containing the same.

Description of the Prior Art

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It is well known that cholesterol causes cardiovascular disease when taken excessively in spite of its vital role of being the constituent of membrane and the precursor of hormone. So far, there is no possible way of preventing this problem but intaking low cholesterol diet. Medicines for hyperlipidemia are effective but have side-effects, as an example, hepatic disorders resulting from the inhibition of the enzyme synthesizing cholesterol. Thus, the use of medicines is extremely limited.

Numerous materials are known for lowering serum cholesterol level in the body such as chitosan, phytosterol, inositol, pectin, etc. However, there is no clear description on the effect or mechanism of the materials except phytosterol. It has been reported that phytosterol, which is a kind of plant sterol, lowers serum cholesterol level by reducing the absorption of cholesterol in the intestines through competition with LDL-cholesterol. In this regard, FDA approved phytosterol as a food additive not affecting the biosynthesis of cholesterol and not having any side-effects mentioned above.

Phytosterol means all the alcohol compounds with steroid moiety found in higher plant and covers stigmasterol, spinasterol, campesterol and sitosterol. Sitosterol has  $\alpha$ ,  $\beta$ ,  $\gamma$ - type. Among all the phytosterols, the cholesterol lowering effect of  $\beta$ -sitosterol(24-ethyl-5 $\alpha$ -cholestene-3 $\beta$ -ol) was proven in an animal test using male rat and in a clinical test as well(see: Sugano, M. et al., *J. Nutr.*, 107:2011-2019, 1977).  $\beta$ -sitosterol ester compounds generated from the substitution of fatty acid are reported as having the same cholesterol lowering effect(see: Mattson, F. H. et al., *J. Nutr.*, 107:1139-1146, 1977). For example, when 2g of  $\beta$ -sitosteryl oleate was given to adult for 5 days, serum cholesterol level was reduced approximately 33%(see: Mattson, F. H. et al., *Am. J. Clin. Nutr.*, 35:697-700, 1982). In addition to cholesterol lowering effect,  $\beta$ -sitosterol is known to be the principal component of *Zea mays* L. which is used for treatment of gingivitis and alveolitis. However, it has a critical demerit preventing it from wide use, i.e., its insolubility against oil and water. This hydrophobic and lipophobic properties only allow them to be formulated as tablet or capsule, while making it difficult and inefficient as food ingredient.

To improve these demerits of  $\beta$ -sitosterol,  $\beta$ -sitostanol(24-ethyl-5 $\alpha$ -cholestane-3 $\beta$ -ol), a hardened form of  $\beta$ -sitosterol, was reacted with edible oil to generate  $\beta$ -sitostanol ester, which was proven to be effective in lowering serum cholesterol when used as an additive for the solid oil product such as margarine(see: WO 92/19640). The product obtained in the above process is the mixture of  $\beta$ -sitostanol esters of saturated and unsaturated fatty acids, since edible oil such as rapeseed oil were used. Therefore, the composition of the products is dependent on the array of fatty acids present in the edible oil used in the reaction. The profile of saturated and unsaturated fatty acids is important in cholesterol lowering effect because saturated fatty acids are known

to depress LDL(low density lipoprotein) receptors and decrease clearance of LDL cholesterol from plasma(see: Hayes, K. C. et al., *Prostaglandins. Leukot Essent Fatty Acids.*, 57(4-5):411-418, 1997). Furthermore, the  
5 hydrogenation of  $\beta$ -sitosterol is required to obtain  $\beta$ -sitostanol itself.

Under the circumstances, based on the previous findings that unsaturated fatty acids or the esterified forms of them have lower melting point than saturated  
10 fatty acids or their esterified derivatives and they are usually liquid at room temperature, the present inventors perceived that the phytosterol or phytostanol esterified with unsaturated fatty acid may be soluble in liquid type of oils. In fact,  $\beta$ -sitosteryl oleate obtained by the  
15 estrification reaction of  $\beta$ -sitosterol with methyl oleate was found soluble in oils and the cholesterol lowering effect of the oil products containing  $\beta$ -sitosteryl oleate was confirmed by animal test(see: Korean Patent laid-open Publication No. 98-7535). However, the above method has  
20 a demerit in a sense that the final product is obtained in a mixture of  $\beta$ -sitosteryl oleate and methyl oleate.

In general,  $\beta$ -sitosteryl oleate can be obtained by the reaction of  $\beta$ -sitosterol and the activated forms of oleic acid, such as oleyl chloride(see: Hartman, L.  
25 *Chem.Rev.*, 58:845-864, 1958) or oleic anhydride(see: Mattson, F. H. et al., *J. Lipid Res.*, 5:374-377, 1964). However, these processes are not suitable for the production of food grades as well as for the commercial scale reaction since the activated forms are very  
30 unstable and toxic chemicals are necessary for the preparation of activated forms. Another possible process for preparing  $\beta$ -sitosteryl oleate is the modification of the transesterification reaction of  $\beta$ -sitostanol and edible oil, which was carried out at 90-120°C using  
35 strong basic catalyst, as described in WO 92/19640. However, this process is also undesirable for the preparation of  $\beta$ -sitosteryl oleate because unsaturated

fatty acids(e.g., oleic acid) are unstable and readily oxidized at high temperature.

#### SUMMARY OF THE INVENTION

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The present inventors have made an effort to manufacture fat-soluble phytosterol or phytostanol derivatives which provide cholesterol lowering effect, while focusing on the role of phytosterol which inhibits the absorption of cholesterol in intestine. However, phytosterol has a critical demerit of insolubility against oil and water. This hydrophobic and lipophobic properties make it difficult and inefficient to be applied for various formulation. Under these circumstances, the inventors synthesized lipophilic derivatives under the mild reaction conditions by the esterification of phytosterol or phytostanol with unsaturated fatty acid. The lipophilic derivatives of phytosterol or phytostanol are soluble in liquid oils such as corn oil and provide a cholesterol lowering effect.

A primary object of the present invention is, therefore, to provide a method for manufacturing fat-soluble phytosterol or phytostanol esters of unsaturated fatty acid under the mild reaction conditions.

The other object of the invention is to provide foodstuffs containing the fat-soluble phytosterol or phytostanol ester of unsaturated fatty acid.

#### DETAILED DESCRIPTION OF THE INVENTION

The method for manufacturing fat-soluble phytosterol or phytostanol ester of unsaturated fatty acid of present invention comprises the steps of: esterification of phytosterol or phytostanol with

unsaturated fatty acid by dissolving them in a nonpolar organic solvent with a basic catalyst and adding a carboxyl group activating agent dissolved in a nonpolar organic solvent; and, precipitation of the esterified  
5 product in methanol or a mixture of methanol and acetone, after filtering the esterified product and evaporating the nonpolar solvent under a reduced pressure.

Further, the final product can be obtained in a wax form by solidifying at  $-2^{\circ}\text{C}$ , discarding the remaining  
10 solvent, and drying the product under a reduced pressure.

In carrying out the method of present invention, toluene, methylene chloride, dichloroethane, tetrahydrofuran, benzene or diethylether is employed as  
15 the nonpolar organic solvent, and 4-dimethylaminopyridine, pyridine or triethylamine as the basic catalyst, 1,3-dicyclohexylcarbodiimide (DCC), 1-ethyl-3-[3'-dimethylaminopropyl]-carbodiimide, oxalyl chloride, carbonyl diimidazole, 2-chloropyridium, 2,2'-dipyridyl  
20 disulfide or 2-imidazolyl disulfide, as the carboxyl group activating agent. Used phytosterol is known as an alcohol compound with steroid moiety discovered in higher plant, which covers stigmasterol, spinasterol, campesterol and sitosterol, preferably sitosterol  
25 including  $\alpha$ ,  $\beta$ ,  $\gamma$ -type; phytostanol includes sitostanol, campestanol which is hardened form of that; and, unsaturated fatty acid to be used has between 4 and 22 carbon atoms, preferably from 12 to 20, more preferably from 16 to 18 and has degree of unsaturation of 1 to 3.

30 The present invention also provides foodstuffs containing the phytosterol or phytostanol ester of fatty acid which is soluble in liquid oil. In this regard, it should be noted that these examples are not meant to limit the scope of foodstuffs, such as, cooking oil,  
35 salad oils, salad dressing, mayonnaise, margarine, chocolate, cream, butter and shortening.

The present invention is illustrated in more detail, where  $\beta$ -sitosterol is employed as a representative example of phytosterol and oleic acid as an unsaturated fatty acid, respectively.

5

Step 1: Esterification of phytosterol or phytostanol with unsaturated fatty acid

$\beta$ -sitosterol and oleic acid are dissolved in a nonpolar organic solvent in a molar ratio of 1:1 to 1:3, preferably 1:2. A basic catalyst is subsequently added, and all particles are mixed completely under the refluxed temperature. Then, a carboxyl group activating agent dissolved in organic solvent is added in a drop-wise for 10 min to 90 min, preferably 30 min to 60 min, most preferably 40 min in a molar ratio of 1:1.1 to 1:3, preferably 1:1.2 to 1:1.5, most preferably 1:1.3, based on the total amount of  $\beta$ -sitosterol in the reaction mixture, and reacted under a refluxed temperature for 10 min to 5 hrs, preferably, 30 min to 3 hrs, most preferably 1hr. Finally, completion of the reaction is confirmed by thin layer chromatography(TLC).

In carrying out the esterification, toluene, methylenechloride, dichloroethane, tetrahydrofuran, benzene or diethylether is employed as the organic solvent; DMAP, pyridine or TEA is employed as the basic catalyst; DCC, 1-ethyl-3-[3'-dimethyl aminopropyl]carbodiimide, oxalyl chloride, carbonyl diimidazole, 2-chloropyridium, 2,2'-dipyridyl disulfide or 2-imidazolyl disulfide is employed as the carboxyl group activating agent, respectively.

Step 2: Preparation of  $\beta$ -sitosterol ester of unsaturated fatty acid

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After the esterification of  $\beta$ -sitosterol and oleic



acid, the reaction mixture is filtered and the solvent is evaporated from the filtrate under a reduced pressure, the residue is mixed with methanol or methanol/acetone mixture(8:2, v/v) violently at the temperature of 30 to 50°C, preferably 35 to 45°C, most preferably 40°C, and precipitated by chilling at -5 to 6°C, for 30 min to 30 hrs, preferably 1 to 10 hrs, most preferably 2 to 5 hrs, finally to give  $\beta$ -sitosterol ester of unsaturated fatty acid, i.e.,  $\beta$ -sitosteryl oleate.

10

Cholesterol lowering effect of the  $\beta$ -sitosteryl oleate was investigated by employing male rat animal test. The fat-soluble ester compound of the present invention can be dissolved in oil, for example, corn oil and sesame oil, and has physical properties like commercially available oil in a sense of quality.

The present invention is further illustrated in the following examples, which should not be taken to limit the scope of the invention. In particular,  $\beta$ -sitosterol and oleic acid are representative examples of phytosterol and unsaturated fatty acid, respectively. Therefore, the present invention can be applied to all kinds of phytosterols such as stigmasterol, spinasterol, campesterol and sitosterol, and all kinds of unsaturated fatty acids such as linoleic acid and linolenic acid having the degree of unsaturation of 1 to 3. Similarly, since corn oil is a representative example of liquid oil, it is obvious that the invention can be applied to all oils such as sesame oil, olive oil, cotton seed oil, soy bean oil, safflower oil, rape seed oil, sunflower oil, peanut oil and rice bran oil.

Example 1: Esterification of  $\beta$ -sitosterol and oleic acid

35

10g(24.11mmol) of  $\beta$ -sitosterol and 13.62g(48.23mmol) of oleic acid were dissolved in 20ml of

methylenchloride in a round bottom flask, 0.24g(1.93mmol) of DMAP, a basic catalyst, was subsequently added, and mixed violently while refluxing under heating, in a water-bath until all particles in the mixture disappear completely. Then, 6.47g(31.35mmol) of DCC, a carboxyl activating agent, which was dissolved in 20 ml of methylenchloride, was added in a drop-wise for 40 min to the mixture of  $\beta$ -sitosterol, oleic acid and DMAP. Then, the reaction mixture was further stirred under a refluxed temperature for 1 hr, and completion of the reaction was confirmed by TLC.

Example 2: Preparation of  $\beta$ -sitosteryl oleate

15 Example 2-1: Precipitation by methanol

After the esterification of  $\beta$ -sitosterol and oleic acid, the reaction mixture was filtered and the solvent was evaporated from the filtrate under a reduced pressure, to give yellowish oil. Then, to the residue was added 100 ml of methanol, stirred vigorously at the temperature of 40°C for 1 hr, and left at -2°C for 2 hrs to solidify the product in a wax form. After discarding the remaining solvent, 100 ml of methanol was added again, and repeated twice to remove oleic acid completely. Finally, 15.16g of  $\beta$ -sitosteryl oleate was obtained(Yield=93%).

Example 2-2: Precipitation by methanol/acetone mixture

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After the esterification of  $\beta$ -sitosterol and oleic acid, the reaction mixture was filtered and the solvent was evaporated from the filtrate under a reduced pressure, to give yellowish oil. The oily material dissolved in 20 ml of methylenchloride was added in a drop-wise in methanol/acetone(8:2, v/v) mixture and precipitated. After keeping it at 4°C for 24 hrs, the resultant was

filtered to obtain  $\beta$ -sitosteryl oleate of wax form (Yield=51%).

Example 3:

5 The effects of fat-soluble ester compound on cholesterol absorption was investigated by employing male rat animal test, and compared with  $\beta$ -sitosterol, which is a representative plant sterol and a well-known  
10 inhibitor of cholesterol absorption, as a positive control. 6 week-old SPF SD male rats were used. Rats were housed in a room equipped with a 12-hour light-dark cycle with free access to tap water and ration during acclimatization for 1 week. They are not given the ration  
15 from 9 AM to 4 PM and were administered 10mg of unlabeled cholesterol suspended in 0.5ml of corn oil and/or 0.025mg of  $^{14}\text{C}$ -cholesterol in 0.08 ml of ethanol for 3 days. They can freely access the ration and water from 3 hrs after the treatment of cholesterol and then are not fed  
20 overnight before being sacrificed. They are anesthetized with diethylether and the blood is collected from the heart. For the experiment, rats are divided into 6 different groups and effects of phytosterol derivative and  $\beta$ -sitosterol were investigated, as shown in Table 1  
25 below. Then, 6 ml of blood were collected from the hearts of rats and centrifuged at 2000xg for 20 min. To quantify the  $^{14}\text{C}$ -cholesterol in the blood, 1.5 ml of supernatant(plasma) was taken and 10 ml of cocktail solution was added. Using a liquid scintillation counter,  
30 radioactivity was counted for 2 min per each sample. All data were represented as mean  $\pm$  SD, and statistical analysis of data was carried out by the Student's t-test. Significance of the value was accepted, when P was <0.05.

35 Table 1: Experimental groups and treatments

Experimental Groups	Background	Negative control	Sitosterol (Positive control)		Fat-soluble derivative	
	Group 1	Group 2	Group 3	Group 4	Group 5	Group 6
Amount of Cholesterol Treated into Rats by p.o.	10 mg cold cholesterol in 0.5 ml corn oil	0.025 mg <sup>14</sup> C-cholesterol in 0.08 ml of ethanol & 10 mg cold cholesterol in 0.5 ml of corn oil				
Sort of Samples	-	-	$\beta$ -sitosterol		LPSS	
Amounts of Samples by p.o.	-	-	30 mg in 1.0 ml corn oil	50 mg in 1.67 ml corn oil	30 mg in 1.0 ml corn oil	50 mg in 1.67 ml corn oil
Numbers of Rats	3	6	5	5	5	5

The rats were treated with 3 $\times$  and 5 $\times$  amounts of phytosterol derivatives and 10mg cholesterol in the main experiment.  $\beta$ -sitosterol reduces the cholesterol absorption by about 30% as shown in Table 2 and the fat-soluble derivative also significantly reduced the cholesterol absorption by about 30% which is almost equally effective as  $\beta$ -sitosterol.

Table 2: Effects of sitosterol, fat-soluble derivative on the exogenous cholesterol absorption.

Groups (treatment)	cpm	% of inhibition
Group 1 (unlabeled cholesterol)	47.3 $\pm$ 19.7	
Group 2 ( <sup>14</sup> C- & unlabeled cholesterol)	74566.4 $\pm$ 4121.7*	
Group 3 ( <sup>14</sup> C- & unlabeled cholesterol and 3 $\times$ $\beta$ sitosterol)	51766.9 $\pm$ 6602.4*	31
Group 4 ( <sup>14</sup> C- & unlabeled cholesterol and 5 $\times$ $\beta$ sitosterol)	52086.5 $\pm$ 2587.2*	30
Group 5 ( <sup>14</sup> C- & unlabeled cholesterol and 3 $\times$ fat-soluble derivative)	52365.7 $\pm$ 10914.4*	30
Group 6 ( <sup>14</sup> C- & unlabeled cholesterol and 5 $\times$ fat-soluble derivative)	48593.7 $\pm$ 4106.9*	35

<sup>a</sup> Values are shown as mean  $\pm$  SD (n=5 or 6).

\* denotes significant differences,  $p < 0.01$ , compared with control(Group 2).

#### 5 Example 4:

The solubility of  $\beta$ -sitosteryl oleate prepared in Example 1 was summarized in Table 3 below. Solubility of sample of each concentration was measured by keeping at each temperature for 3 days, whose results were shown in Table 4. In Table 3, 0 represents the condition of soluble and X, the formation of precipitant, respectively. As can be seen in Table 3,  $\beta$ -sitosteryl oleate was soluble in liquid oil. When the oil containing 1%(v/v)  $\beta$ -sitosteryl oleate was compared with a common oil according to the KFDA standard, there was no significant difference in a sense of quality(see: Table 4).

20 Table 3: Solubility difference of fat-soluble ester compound

	1%	2%	3%	4%	5%	7%	10%
4°C	0	0	0	0	0	0	X
25°C	0	0	0	0	0	0	0
37°C	0	0	0	0	0	0	0

25 Table 4: Comparison of oil containing 1%  $\beta$ -sitosteryl oleate with a common oil

	Standard	Common corn oil	Added corn oil
Acid value	<0.6	0.085	0.085
Oxide value	<1.0	0.1	0.12
Saponification value	<187~195	192	194
Unsaponification value	<2.0	0.5	2.08
Iodine value	103-130	127	127.4

As clearly illustrated and demonstrated as above,  
the present invention provides a method for manufacturing  
fat-soluble phytosterol or phytostanol ester of  
unsaturated fatty acid for inhibiting the intake of  
5 cholesterol and foodstuffs containing the same.

Although the preferred embodiments of the present  
invention have been disclosed for illustrative purpose,  
those who are skilled in the art will appreciate that  
10 various modifications, additions and substitutions are  
possible, without departing from the scope and spirit of  
the invention as disclosed in the accompanying claims.

WHAT IS CLAIMED IS:

1. A method for manufacturing fat-soluble ester of  
phytosterol or phytostanol unsaturated fatty acid, which  
5 comprises the steps of:
  - i) esterification of phytosterol or phytostanol  
with unsaturated fatty acid by dissolving them in a  
nonpolar organic solvent with a basic catalyst and adding  
a carboxyl group activating agent dissolved in a nonpolar  
10 organic solvent; and,
  - ii) precipitation of the esterified product in  
methanol or a mixture of methanol and acetone, after  
filtering the esterified product and evaporating the  
nonpolar solvent under a reduced pressure.  
15
2. The method for manufacturing fat-soluble ester  
of phytosterol or phytostanol unsaturated fatty acid of  
claim 1, wherein the phytosterol is selected from the  
group consisting of stigmasterol, spinasterol,  
20 campesterol and sitosterol.
3. The method for manufacturing fat-soluble ester  
of phytosterol or phytostanol unsaturated fatty acid of  
claim 1, wherein the phytostanol is sitostanol or  
25 campestanol.
4. The method for manufacturing fat-soluble ester  
of phytosterol or phytostanol unsaturated fatty acid of  
claim 1, wherein the unsaturated fatty acid has 4 to 22  
30 carbons and degree of unsaturation of 1 to 3.
5. The method for manufacturing fat-soluble ester  
of phytosterol or phytostanol unsaturated fatty acid of  
claim 1, wherein the nonpolar solvent is selected from  
35 the group consisting of toluene, methylenechloride,  
dichloroethane, tetrahydrofuran, benzene and diethylether.

6. The method for manufacturing fat-soluble ester of phytosterol or phytostanol unsaturated fatty acid of claim 1, wherein the basic catalyst is selected from the group consisting of 4-dimethylaminopyridine (DMAP),  
5 pyridine and triethylamine (TEA).

7. The method for manufacturing fat-soluble ester of phytosterol or phytostanol unsaturated fatty acid of claim 1, wherein the carboxyl group activating agent is  
10 selected from the group consisting of 1,3-dicyclohexylcarbodiimide (DCC), 1-ethyl-3-(3'-dimethylaminopropyl)-carbodiimide, oxalyl chloride, carbonyl diimidazole, 2-chloropyridium, 2,2'-dipyridyl disulfide and 2-imidazolyl disulfide.

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8. The method for manufacturing fat-soluble ester of phytosterol or phytostanol unsaturated fatty acid of claim 1, wherein the esterification is carried out by employing phytosterol or phytostanol and unsaturated  
20 fatty acid in a molar ratio of 1:1 to 1:3.

9. The method for manufacturing fat-soluble ester of phytosterol or phytostanol unsaturated fatty acid of claim 1, wherein the esterification is carried out by  
25 adding the carboxyl group activating agent in a drop-wise to phytosterol or phytostanol in a molar ratio of 1:1.1 to 1:3 under a refluxed temperature for 1 to 5 hours.

10. The method for manufacturing fat-soluble ester  
30 of phytosterol or phytostanol unsaturated fatty acid of claim 1, wherein the precipitation is carried out at the temperature of -4 to 6°C for 1 to 30 hrs.

11. The method for manufacturing fat-soluble ester  
35 of phytosterol or phytostanol unsaturated fatty acid of claim 1, which is soluble in liquid oil selected from the group consisting of corn oil, olive oil, coconut oil,



cotton seed oil, soy bean oil, rice bran oil, safflower oil, rapeseed oil, sunflower oil, sesame oil and peanut oil.

- 5           12. Foodstuffs containing fat-soluble ester compound of phytosterol or phytostanol unsaturated fatty acid which are manufactured by the method of claim 1.

- 10           13. The foodstuffs of claim 12, which are vegetable oil, cooking oil, salad oil, salad dressing, mayonnaise, margarine, chocolate, cream, butter or shortening.

# INTERNATIONAL SEARCH REPORT

International application No.  
PCT/KR 99/00569

## A. CLASSIFICATION OF SUBJECT MATTER

IPC<sup>7</sup>: C 09 J 9/00, A 23 L 1/24

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC<sup>7</sup>: C 09 J 9/00, A 23 L 1/24

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

STN-CAS; WPIL

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P,X	WO 99/56558 A1 (RAISIO BENECOL OY) 11 November 1999 (11.11.99), claims.	1-13
A	US 5117016 A (T.L. TACKETT et al.) 26 May 1992 (26.05.92), column 3, lines 1-51; claims.	1-5,10
P,A	WO 99/25362 A1 (HENKEL) 27 May 1999 (27.05.99), claims.	1-4,12,13
A	WO 92/19640 A1 (RAISION MARGARINI) 12 November 1992 (12.11.92), claims.	1,12,13
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☐ Further documents are listed in the continuation of Box C.

☒ See patent family annex.

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# INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No.

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